GROWTH AND CALCIUM METABOLISM OF EMBRYOS OF THE LONG-NECKED TORTOISE, *CHELODINA LONGICOLLIS* (SHAW).

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Growth (change in mass) and calcium metabolism of embryonic turtles have received little attention. The present study extends this small data set to include the long-necked tortoise, *Chelodina longicollis*, Shaw (Testudinata: Chelidae). Eggs were 3/4 buried in vermiculite and incubated under controlled moisture (-200kPa) and temperature (30°C) conditions. Embryonic growth was described by the regression of log dry mass on log day of incubation. Total calcium in the egg did not change but was redistributed. The demand for calcium during embryogenesis exceeded the amount available from the yolk and albumen; the additional calcium required for osteogenesis was supplied by the shell. □ *Growth, calcium metabolism, tortoise embryos.*

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The few studies that have considered growth (change in mass) of embryonic turtles have been restricted to cryptodires (Ackerman, 1980, 1981, Cheloniidae: *Caretta, Chelonia*; Miller, 1982, Cheloniidae: *Caretta, Chelonia, Eretmochelys, Natator*; Morris et al., 1983, Chelydridae, *Chelydra serpentina*; G. Packard et al., 1983, Emydidae, *Chrysemys picta*). Growth of these embryos follows an exponential or logistic form. There are no published data on the growth of embryonic pleurodiran turtles.

Four studies have considered the pattern of calcium metabolism within incubating eggs of oviparous reptiles (Packard et al., 1984a, Chelydridae: *Chelydra serpentina*; Packard et al., 1984b, Colubridae: *Coluber constrictor*; Packard et al., 1985, Agamidae: *Amphibolurus barbatus*; Packard and Packard, 1986, Emydidae, *Chrysemys picta*). Other studies concerning the utilization of calcium in reptilian eggs only identified that the shell was the primary source (Simkiss, 1962, 1967; Bustard et al., 1969: Dermochelyidae, Cheloniidae). In eggs of the American snapping turtle, *Chelydra serpentina*, about 56% of the calcium for embryogenesis originates in the shell (Packard et al., 1984a); embryos of sea turtles obtain between 60 and 80% of the required calcium from the shell (Simkiss, 1962, 1967; Bustard et al., 1969).

The present study was designed to extend the knowledge of growth and calcium metabolism in reptilian embryos to include the long-necked tortoise, *Chelodina longicollis* (Shaw, 1794) (Testudinata: Chelidae). The specific objectives were (a) to describe the pattern of embryonic growth, (b) to determine the amount of calcium in each of the compartments of the egg (yolk, albumen, shell) at oviposition and (c) to describe the pattern of calcium utilization and the relative contribution from these sources during development of the embryo. Preovulatory follicles were also analyzed for calcium content.

METHODS

Gravid tortoises were collected by netting dams between 13 November and 7 December, 1983 at Herbert Park (30°27'S, 151°50'E), approximately 25 km northeast of Armidale, NSW. Tortoises were housed in individual aquaria at ambient temperature (18-25°C). No tortoise was retained longer than 10 days.

The straight carapace length (SCL) of each tortoise was measured to the nearest 0.01cm with calipers from the anterior edge of the nuchal (cervical) scute to the posterior edge of the postcentrals (12th marginals). Each tortoise was weighed to the nearest 5 grams using a Pesola spring balance.

Tortoises were palpated in the inguinal area to determine if eggs were present. Oviposition was

induced by intracoelomic injection of oxytocin at a dosage of 1 iu/100g of total body mass (Ewert and Legler, 1978). If the first injection did not produce eggs within one hour, a second injection was given. If no eggs were expelled, a third injection was given. No more than three injections were given to any tortoise; all eggs were laid in water.

Within 2 minutes of egg laying, eggs were removed from the water, wiped dry, and numbered using permanent ink. Eggs were weighed to the nearest 0.01 gram on a Bosch p115 top pan balance and measured (length and width) to the nearest 0.01cm with calipers.

Eggs were incubated in 2.5 litre plastic containers sealed with tight-fitting lids. Eggs were 3/4 buried in vermiculite moistened to 15 % water by weight (approximately -200kpa). The moisture level was maintained by adding 3-8ml distilled water every 7-10 days during incubation. The temperature was maintained at 30+/- 0.7°C throughout incubation. The position of each container was regularly shifted within the incubator to minimize the potential effects of temperature gradients (Bull et al., 1982).

If most eggs in a clutch did not exhibit a white area on the uppermost part of the shell (an indication of viability) within 7 days of oviposition, the clutch was not used for the calcium metabolism experiments.

One egg from each clutch was collected at predetermined times during incubation (0, 20, 30, 35, 40, 45, 50, 55, 60 days, and hatching). Each egg was re-weighed and re-measured at the time of sampling then opened and the contents were separated into embryo, yolk, membranes and fluids, and shell. The fresh mass of each component was obtained to the nearest milligram. The embryos were staged according to Yntema (1968). To determine hatching success, it was assumed that normal embryos obtained from eggs sampled during incubation would have developed into normal hatchlings and it was assumed that abnormal embryos would not have produced hatchlings.

Follicles (n=66) of different measured diameters (±0.01cm) were removed from the ovaries of four decapitated tortoises and weighed (± 0.01g).

Each egg component and follicle was dried to constant mass at 60°C. Embryos were ground in a mill into fine particles; yolks and follicles were homogenized by hand using a mortar and pestle. Subsamples of 300mg or entire samples were digested in boiling, concentrated nitric acid aided by 30 % hydrogen peroxide (both reagent grade). Samples were brought to 100ml volume with distilled water. Further dilutions of digestate were made using 0.02 % strontium chloride (1ml sample + 9ml dilutent) dispensed through an auto-dilution system (Hook and Tucker Instruments, New Addington, England). Five samples of water from vermiculite that had been soaked for 20 days in distilled water were also analyzed for calcium.

The calcium content of each sample was determined using a Pye Unicam SP 190 single-beam Atomic Absorption Spectrophotometer (using an air/acetylene flame) coordinated with an SP 450 automatic sample changer following standard procedures.

The data were analyzed using one-way analysis of variance with the initial egg mass as the potential covariate (programme BMDP2V, Dixon et al., 1981). Further examination of the data for calcium in yolks and embryos was done by comparing the regressions for the variables among the clutches. This approach was taken because only one egg was collected from each clutch at the sampling times which precluded use of analysis of co-variance with groupings by clutch and day of sampling. Data for the regression analysis were truncated to eliminate time zero because preliminary analysis demonstrated no significant differences occurred between data at time zero and day 20. Truncation simplified the curvilinear nature of the remaining data and allowed better comparison on a linear basis. Because of this, however, these regression lines do not fully describe the entire data set. Sample sizes vary among the treatments; $P = 0.05$ was used to establish significance. Least Significant Difference (LSD) values were calculated using Statistix (Analytical Software, St Paul, MN, USA) computer program; calculation of other statistical tests followed Zar (1974).

RESULTS

A total of 64 female tortoises were captured and injected with oxytocin; only 16 (25%) produced eggs (Table 1), usually after a third injection. The general effectiveness of oxytocin on the tortoises was low. The time interval between injection and oviposition was variable. The shortest period was 1.5 hours and the longest was nearly 15 hours. All tortoises required at least two injections before oviposition was induced. Most tortoises were not obviously distressed by handling or injection; all animals

TABLE 1. Summary on tortoises, clutches, incubation period, and hatching success.

Female Number	Weight (g)	SCL (cm)	Number of eggs	Total Clutch Weight (g)	Percent Clutch Weight (g)	Incubation Period (days)	Hatching Success (%)
Used in Calcium Determination							
6A	1020	19.34	14	74.68	7.32	81	92.8
11	1020	19.97	12	94.01	9.02	75.5	91.6
16	750	17.94	8	38.76	5.16	64	100
27	1010	19.15	17	84.17	8.33	80	70.5
41	790		9	55.69	7.05	74	100
61	1060		11	74.68	7.05	67	100
82			11	62.08		71.5	100
89	780		9	49.51	6.35	66.5	100
122	914	19.43	11	79.59	8.71	70	100
Mean	918	19.17	11.33	68.13	7.37	72.17	94.99
Std Dev	118.9	0.671	2.624	16.815	1.203	5.622	9.229
Minimum	750	17.94	8	38.76	5.16	64	70.5
Maximum	1060	19.97	17	94.01	9.02	81	100
Number	8	5	9	9	8	9	9
Not Used in Calcium Determination							
6B	940	19.34	15	67.5	7.18	0	0
15	720	17.84	9	45.28	6.28	0	0
78	980	19.81	4	34.2	3.48	0	0
99			13	89.93		0	0
102	750	17.72	9	62.49	8.33	74.5	22.2
103	1080	20.18	11	66.95	6.2	0	0
118	930	19.25	12	60.89	6.54	0	0
Mean	900	19.023	10.43	61.034	6.33		
Std Dev	126.62	0.932	3.288	16.397	1.466		
Minimum	720	17.72	4	34.2	3.48		
Maximum	1080	20.18	15	89.93	8.33		
Number	6	6	7	7	6		

remained watchful and active during their captivity. Oviposition was usually preceded by a slight increase in activity.

In total, 175 eggs were induced from 16 tortoises giving a mean clutch size of 10.93 eggs (sd=3.47, range=4-17) (Table 1). Non-viable eggs were found in 7 clutches containing 73 eggs.

Size and Weight of Female Tortoises

The mean straight carapace length (SCL) of female tortoises captured during the study was 19.1cm (sd=2.229, Range=14.1-26.5, n=59). There was no significant difference between the mean SCL of tortoises that laid eggs (19.1cm, sd=0.867, range=17.72-20.18, n=11) and the mean SCL of those that did not (19.1cm, sd=2.411, range=14.12-26.5, n=48) (t=0.0831, df=57). In the group of tortoises that yielded eggs, there was no significant difference between the SCL measurements of those tortoises whose eggs were used in the calcium experiment and the others (t=0.254, df=10).

The mean mass of all female tortoises was 855.4g (sd=229.7, range= 364-1440, n=62). There was no significant difference between the mean mass of the tortoises which yielded eggs (909g, sd=130.2g, range=720-1080, n=14) and the mean mass of those that did not (824.5g (sd=266.1, range=364-1440, n=48) (t=1.162, df=61). There was no significant difference between the masses of those females whose eggs were used in the calcium experiment and the others (t=0.252, df=12).

Further comparisons between the tortoises used in the calcium experiment and the others that oviposited revealed no significant differences in the number of eggs (t=1.344, df=14), total clutch weight (t= 1.5889, df=14), or in the percentage of the female weight represented by the total clutch weight (t=1.346, df=12).

The relationship between the straight carapace length and mass of all female tortoises was described by the equation: SCL = 0.00835 female mass + 11.983, R^2=0.86 where SCL is in cm and weight is in grams. The relationship between SCL and log of the mass for the tortoises which yielded eggs was described by the equation: SCL (cm) = 21.597-0.3572 log female mass (g), R^2=0.26.

Size, and Weight of Eggs

Based on the 102 viable eggs which were used in the study of calcium metabolism, the mean mass of freshly oviposited eggs grouped by clutch was 6.0g (sd=1.04, range=4.85-7.83, n=9 clutches). The mean length of these eggs was 3.09cm (sd=0.1824, range=2.81-3.38, n=9 clutches); the mean width was 1.82cm (sd=0.16, range=1.51-2.04, n=9 clutches).

The relationships between egg length, width and mass were described best by the equations:

Mean Egg Width = -0.31621 (Mean Egg Length) + 2.7797,

R^2=0.074, P>0.05;

Mean Egg Width = 0.14245 (Mean Egg Fresh Mass) + 0.9742,

R^2=0.725, P<0.01

Mean Egg Length = 0.03018 (Mean Egg Fresh Mass) + 2.8811,

R^2=0.037, P>0.05.

There was a tendency for the width of eggs to increase as mass increased, but the mean egg length did not increase proportionally.

Female mass was not correlated with the number of eggs produced (R^2= 0.099, P>0.05) but was slightly correlated with mean egg mass (R^2= 0.247, P<0.05) (Fig. 1). The poor correlation between egg mass and female mass is partially explained by the ineffectiveness of the oxytocin on the tortoises; it is possible that some females were induced to oviposit incomplete clutches. The correlation between female SCL and total clutch mass was also poor (R^2=0.269) and so was that between female mass and total clutch mass (R^2=0.512). The mass of clutches that contained viable eggs represented an average of 7.4 % (range= 5.16-9.02, n=9) of the female mass.

There was a slight decrease in the mass of all eggs during incubation (egg mass = -0.042 + 6.863 days, n=102). The slopes of the regressions of the change in egg mass grouped by clutch were not significantly different (F 0.05 [8,62]=1.123); however the elevations were (F 0.05 [8,70]=56.64). Analysis of variance among the initial masses of the eggs from different clutches showed that significant differences occurred between clutches (Table 2).

Among the 102 eggs used in the calcium study, 95 eggs (93.1 %) produced normal embryos or hatchlings (given the assumptions above); only 6.9 % of these eggs failed to develop normally.

The mean duration of incubation at 30°/. 0.7°C was 70.41 days (sd=5.85, range=63-82, n=12 eggs from 7 clutches) when averaged from the clutch averages. When considered independently of clutch, the mean was 69.2 days (sd=5.25, range 63-82, n=12).

Wet and Dry Mass

The relation between wet mass and dry mass of follicles followed the power curve (log dry mass = 0.9608 + 0.6693 log wet mass, R^2=0.993). The diameter and wet mass of follicles were related according to the equation: log wet mass = 2.896 + 0.4514 log diameter, R^2=0.871.

There was no significant difference between the mean mass of the 15 largest follicles and the mean mass of yolks of ovipositional eggs (wet mass: t=1.13, df=22, P>0.05). However, there was a significant difference between the dry masses (t=3.71, df=22, P<0.05). The mean dry mass of the follicles was heavier than that of the ovipositional egg yolks, this may have resulted from differences between clutches.

At oviposition, a turtle egg is comprised of shell, albumen, yolk and embryo. However, the embryo is at the early gastrula stage (Yntema, 1968; Cunningham, 1922; Lynn and von Brand, 1945; Miller, 1985) and contributes little to the total mass. Because of the difficulty of removing the blastodisc from the yolk, its actual contribution was ignored until it could be retrieved (about Stage 10, Yntema, 1968). The proportions of the egg components changed little during the first third of incubation but thereafter the amount of yolk and albumen decreased and the amount of embryo increased (Table 3).

The mass of the fresh egg shell decreased significantly during incubation (F 0.05[10,57] =3.885) but the dry mass of the shells did not decrease significantly (F 0.05[6,28] = 0.186).

Although there was a significant decrease in

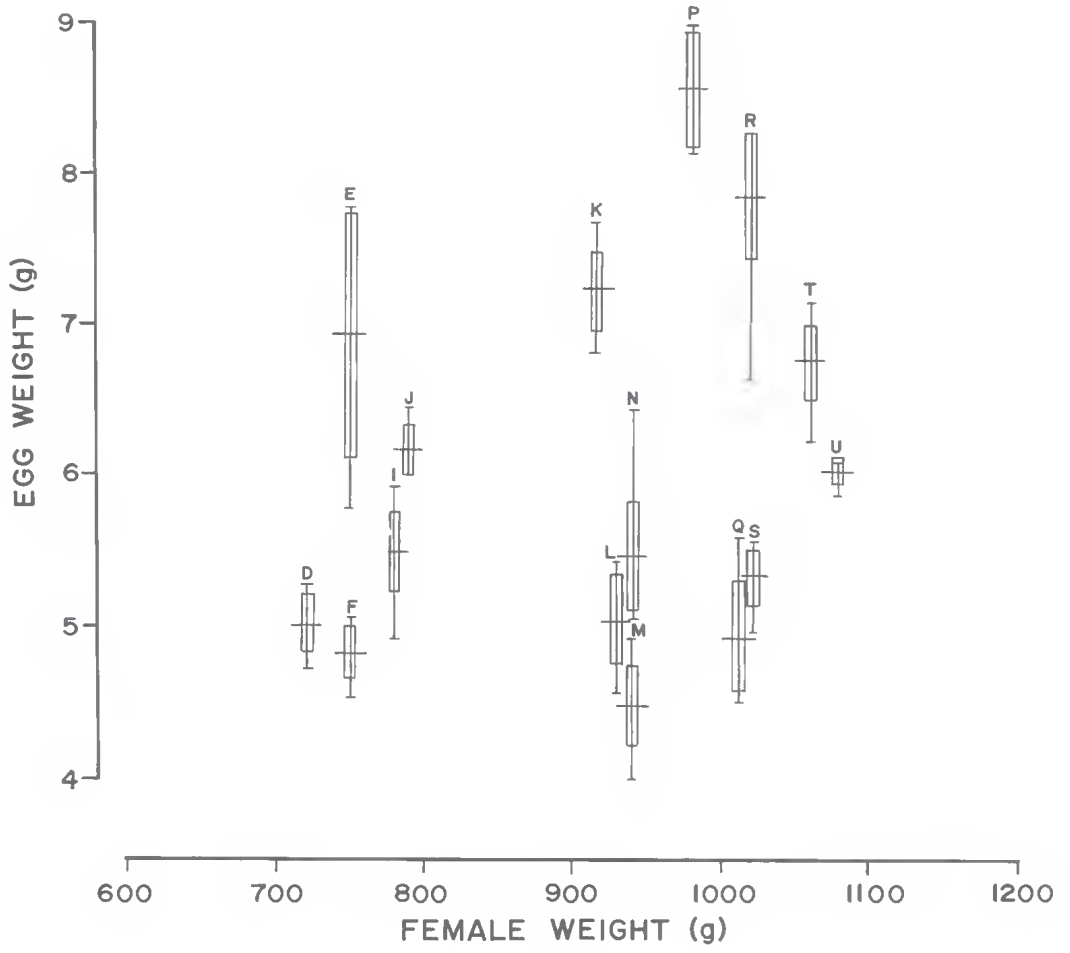

FIG. 1. The relation between female body weight and mean egg weight grouped by clutch. Letters identify clutches: D=15, E=102, F=16, H=82, I=89, J=41, K=122, L=118, M=6b, P=78, Q=27, R=11, S=6a, T=61, U=103.

the wet mass of the albumen beginning at about one-third of the incubation period (F 0.05[8,29] = 6.265), there was not a significant decrease in the dry mass of the solid material of the albumen during incubation (F 0.05[8,29] = 2.201).

The wet mass of the yolk decreased significantly during incubation (F 0.05[10,55] = 22.866). This was mirrored by the decrease in dry mass of the yolks (F 0.05[10,55] = 13.277) (Fig. 3).

The combined mass of the fresh yolk and albumen decreased significantly during incubation (F 0.05[9,42] = 15.464). The combined mass of dry yolk and albumen exhibited a significant decrease (F 0.05[9,42] = 26.416).

The mass of water in the albumen, yolk and combined yolk and albumen decreased during incubation in concert with the decrease in the total mass of each. However, the percentage of water in the albumen and yolk remained relatively constant (Table 4). Water comprised approximately 95.3% of the mass of fresh albumen throughout incubation. Water contributed approximately 69.3 % of the total mass of the yolk at the beginning of incubation but only 56.3 % at hatching. There appeared to be little change in the proportion of water in the yolk during the first

TABLE 2. Results of analysis of variance between clutches for ovipositional weight, length, and width of eggs.

	Sum of Squares	Degrees Freedom	Mean Square	F	P
Ovipositional Egg Weight					
Regression	103.561	8	12.945	154.597	0.001
Residual	7.787	93	0.984		
Ovipositional Egg Length					
Regression	3.115	8	0.389	53.943	0.001
Residual	0.671	93	0.007		
Ovipositional Egg Width					
Regression	2.097	8	0.262	15.821	0.001
Residual	1.541	93	0.017		

one-half of incubation but during the last half, the proportion of water decreased. Even though there was a nett decrease in the solid material in the yolk, the percentage of solids increased because, proportionally more water than solids was removed.

The fresh mass of the embryos increased significantly ($F\ 0.05[9,61] = 24.157$) during incubation. In embryos, as the amount (mass) of water increased, the percentage of water decreased (Table 4). The dry mass of embryos increased significantly during incubation ($F\ 0.05[9,60] = 20.588$).

Stage of development

The stage of development reached by the embryos at any given time during incubation at 30°C was described by the equation: log stage = 1.448 + 0.4325 log days, R^2=0.651. The stages of development increased rapidly during the first half of incubation and began to plateau shortly thereafter (Fig 2). This is predictable because more stages are defined to occur during this

TABLE 3. Percent composition of fresh egg components at selected times during incubation. ND=not determined.

	Day of Incubation				
	0	20	35	50	70
Percent Albumen	42.8	45.9	48.2	23.5	25.3
Weight of Albumen					
Mean	2.26	2.85	2.8	1.32	1.19
Std Dev	0.457	0.965	0.359	0.196	0.499
Number	6	3	3	3	2
Percent Yolk	57.2	52.3	38.2	29.2	2.6
Weight of Yolk					
Mean	3.02	3.25	2.22	1.65	0.12
Std Dev	1.678	0.703	1.16	1.192	0.176
Number	9	3	2	6	11
Percent Embryo	ND	1.78	13.6	47.3	72.1
Weight of Embryo					
Mean	ND	0.11	0.79	2.65	3.37
Std Dev		0.060	0.179	0.596	1.037
Number		5	6	7	12

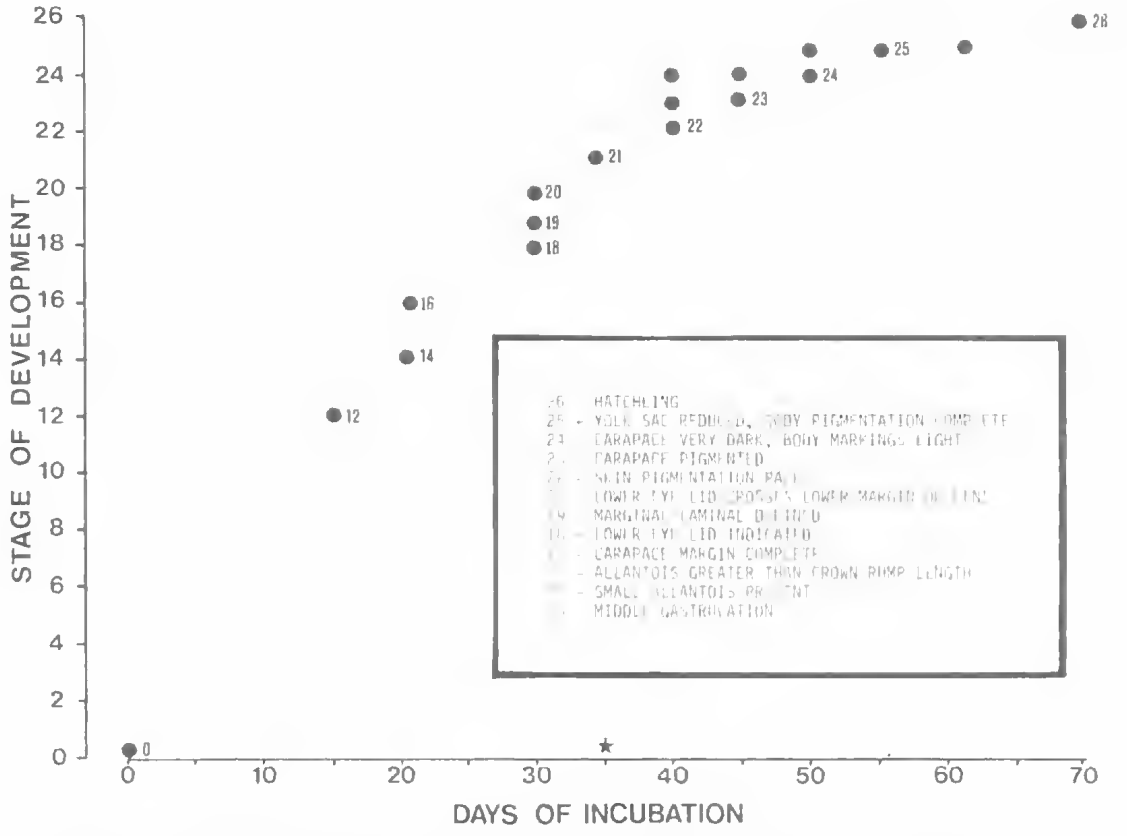

FIG. 2. Stages of development of *Chelodina longicollis* based on the table of normal development for *Chelydra serpentina* (Yntema, 1968). The box provides some of the characteristics for determining the stage.

period when differentiation is occurring rapidly (see Yntema, 1968). Development of the allantois began before the mid-point of incubation. As the membrane grew into position to participate fully in metabolic activity, embryonic growth accelerated.

Calcium in Follicles and Eggs

The calcium available from the incubation medium (vermiculite + distilled water) averaged 0.019mg (range=0.025-0.015, n=5). The pattern of increase in calcium in follicles of increasing dry mass followed the formula:log Ca = 1.3483+ 1.2086 log dry follicle mass (R^2=0.792). The amount of calcium contained in the 15 largest follicles did not differ significantly from that in ovipositional yolks (t = 0.268, P > 0.05).

Twelve samples of shell were analyzed to provide an estimate of total calcium available for translocation into the developing embryo. Shells were collected from early in incubation because later shells tended to fragment. The mean of the samples was 184.9mg Ca (sd=41.054, range=135.6-248.7).

Eighteen samples of albumen were analyzed to determine calcium content. The mean was 0.6252mg Ca (sd=0.4422, range=0.119-1.637). The samples were accepted only as an indication and were not subjected to further analysis because of the potential of contamination by other materials (e.g. blood, extra-embryonic fluids and granules of shell) during preparation.

The amount of calcium in the yolk did not change significantly during the first 30 days of incubation, then it declined rapidly for about 20 days after which it leveled off at about 2% of the mean starting value (Fig. 4; Table 5). Analysis of variance with initial egg mass as the potential covariate showed there was significant decrease in the amount of calcium in the yolk (F 0.05[9,69] = 135.40). Comparison of the slopes of regression lines of the amount of calcium in the yolk against time for each clutch were not

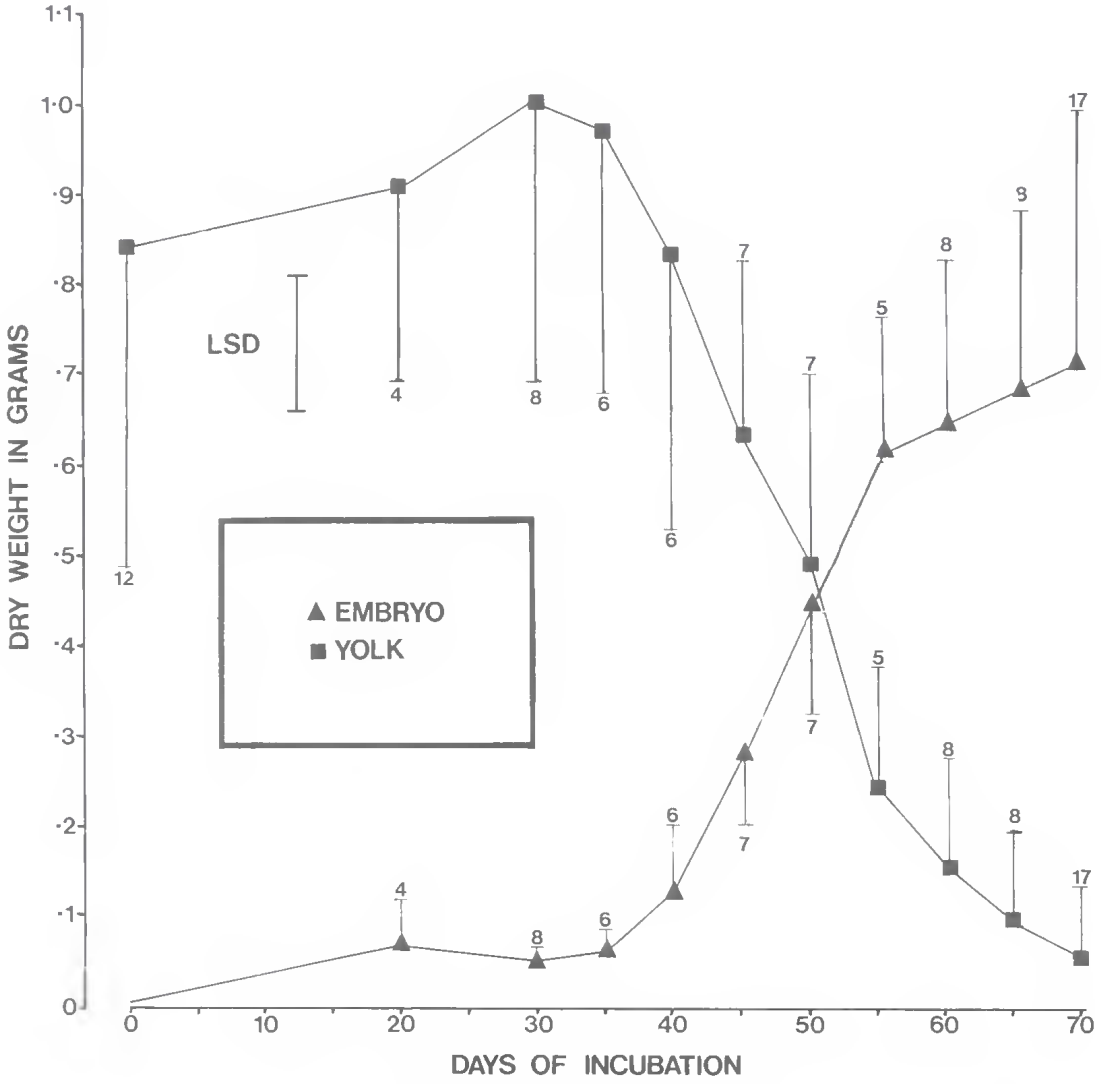

FIG. 3. The change in dry mass of embryos and yolks during incubation of *Chelodina longicollis* eggs. Vertical lines indicate one standard deviation; numbers are sample sizes. Means that are separated by at least one LSD bar are significantly different at the 0.05 level.

significantly different (F 0.05[8,50] = 1.594) but the elevations were (F 0.05[8,58] = 3.058).

The concentration of calcium in the yolk remained relatively stable for the first half of incubation but decreased thereafter to hatching (Fig. 5). Analysis of variance with initial egg mass as the potential covariate revealed that the concentration of calcium in the yolk decreased significantly during incubation (Table 6). Different clutches displayed significantly different slopes indicating that the concentration of calcium during incubation was significantly different (Table 6).

The amount of calcium in the embryos did not increase significantly until after 30 days of development. The rate of change was slow at first then increased rapidly until just before hatching. The amount of calcium in the embryo of ovipositional eggs was assumed to be negligible; this was confirmed by analysis of slightly older embryos (Table 5). Analysis of variance with initial egg mass as the potential covariate demonstrated that significant differences oc-

TABLE 4. Percentage of water and solids in egg components at selected times during incubation. ND= not determined.

	Day of Incubation				
	0	20	35	50	70
Percent Water					
Albumen Mean	95.3	95.4	96.1	96.4	95.3
Std Dev	7.46	1.12		1.34	0.65
Number	6	3	1	3	4
Yolk Mean	69.3	78.6	79.6	63.4	56.3
Std Dev	7.38	3.93		12.3	8.52
Number	9	3	1	6	11
Embryo Mean	ND	94.8	90.1	82.8	78.8
Std Dev		2.8	0.85	1.38	1.62
Number		4	6	7	12
Percent Solids					
Albumen Mean	4.6	4.3	3.9	3.6	4.7
Std Dev	0.746	1.25		1.345	0.65
Number	6	3	1	3	4
Yolk Mean	30.4	21.4	20.4	36.6	43.7
Std dev	7.375	4.05		12.32	8.532
Number	6	3	1	6	11
Embryo Mean	ND	6.6	9.7	17.1	20.8
Std Dev		0.495	0.854	1.384	1.798
Number		2	6	7	13

curred in the amount of calcium in the embryos during incubation (Table 6).

The concentration of calcium in the embryos increased steadily from about 20-30 days until hatching (Fig. 5). The increased variance following 60 days of incubation resulted from either of two sources: (1) contamination by extra-embryonic fluids, blood, or granules of shell or (2) non-discrimination between late stage embryos and hatchlings. Analysis of variance with initial egg mass as the potential covariate substantiated that the concentration of calcium increased significantly during incubation (Table 6).

The total amount of calcium in the egg, excluding the shell, increased significantly during incubation (Table 6). The pattern of change in the total calcium followed that of the yolk for slightly more than the first half of incubation and followed the increase in the embryo thereafter (Fig. 5). The demand for calcium by the embryo for osteogenesis exceeded the amount available from the yolk and albumen combined. Because only a low concentration of calcium was available from the incubation medium and because eggs showed a net loss of water during incubation, it is assumed that the extra requirement was supplied by the shell.

DISCUSSION

References to studies of the ecology and general biology of *Chelodina longicollis* are provided by Cogger et al. (1983); Parmenter (1976) studied the ecology of this tortoise in the Armidale region and provided a comparative review of the older literature.

Parmenter (1976, 1985) found that larger females tended to lay more eggs than smaller ones. The average number of eggs reported was 13.9 (sd=4.29, range 6-23, n=74). The average clutch size in the present study was 10.93 (range 4-17) eggs. Both of these values are consistent with other reports on the number of eggs per clutch (Harrington, 1933: n=up to 20; Goode, 1967: n=10-15; Krefft, 1865: n=15-20; McCooey: 1887, n=15-36; Lucus and Le Souef, 1909: n=7-21; Vestjens, 1969: n=13-24).

At an incubation temperature of 30°C, incubation requires 60-69 days (Parmenter, 1976, 1985), 73-78 days (Goode and Russell, 1968), 53-76.5 days (Legler, 1985) and 63-82 (this

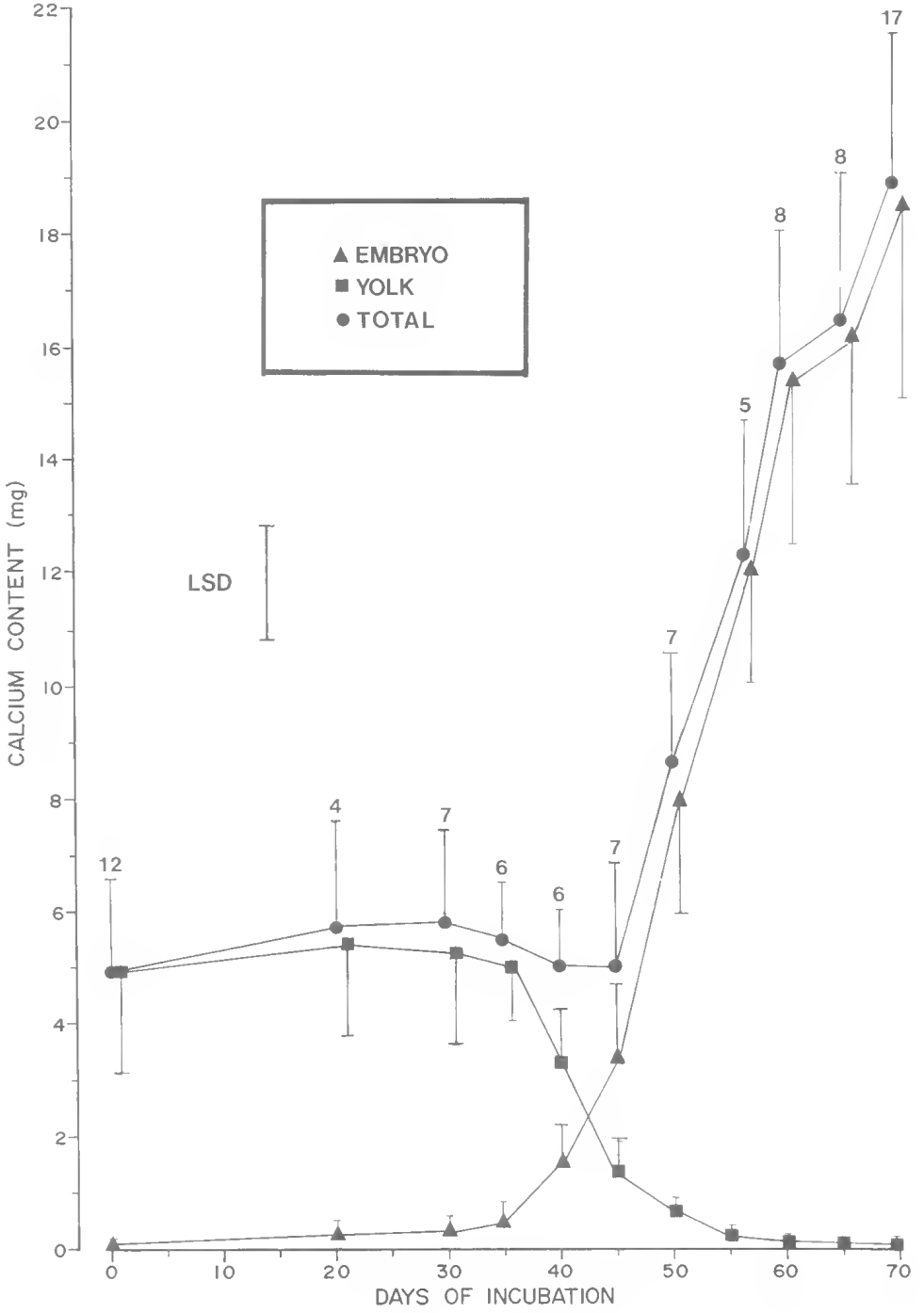

FIG. 4. The content of calcium in embryos, yolks and total egg (excluding the egg shell) during incubation of *Chelodina longicollis* eggs. Vertical lines indicate one standard deviation; numbers are sample sizes. Means that are separated by at least one LSD bar are significantly different at the 0.05 level.

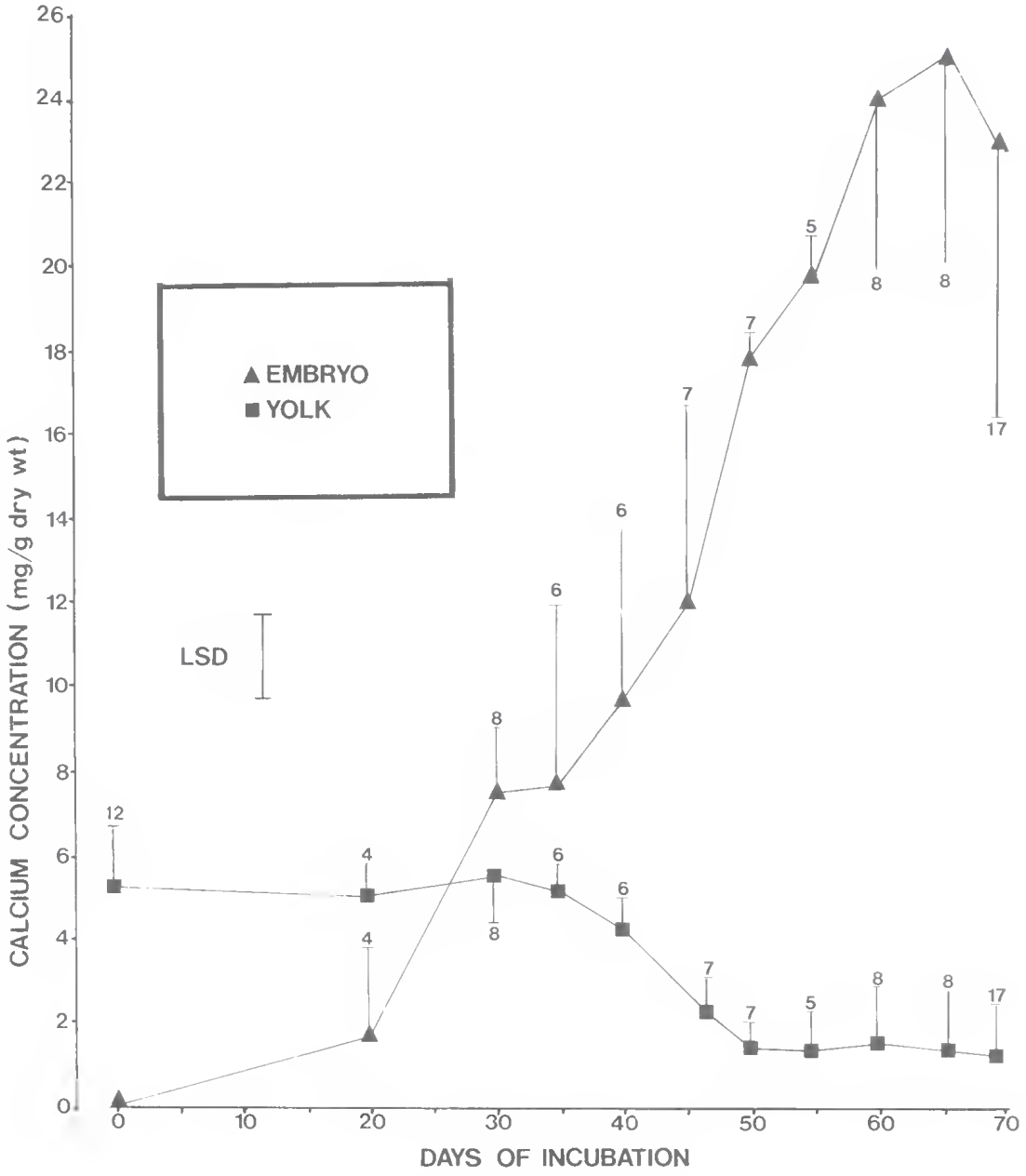

FIG. 5. The concentration of calcium in embryos and yolks during incubation of *Chelodina longicollis* eggs. Vertical lines indicate one standard deviation; numbers are sample sizes. Means that are separated by at least one LSD bar are significantly different at the 0.05 level.

study). Because the duration of incubation varies inversely with temperature, eggs incubated under natural conditions require longer to hatch (Goode, 1967: 130-137d; Goode and Russell, 1968: 131-145d; Vestjens, 1969: 118-150d; Parmenter, 1976: 105-123d).

Parmenter (1976) reported a very strong correlation between the log of female mass and straight carapace length (SCL) (R^2=0.97). In the present study, the linear regression had the best correlation between female mass and SCL. Parmenter (1976) also found a significant, positive

TABLE 5. Amounts of calcium (mg) in egg components at selected times during incubation. ND=not determined.

	Day of Incubation				
	0	20	35	50	70
Albumen Mean	0.625	0.205	0.394	0.227	0.412
Std Dev	0.442		0.321	0.152	0.132
Number	4	1	2	2	5
Yolk Mean	5.232	5.395	5.134	0.758	0.126
Std Dev	1.889	2.246	1.679	0.405	0.128
Number	12	4	6	7	12
Embryo Mean	ND	0.343	0.657	8.192	18.45
Std Dev		0.589	0.117	2.196	3.918
Number		3	5	7	13
Shell Mean	189.66	179.9	160.59		
Std dev	49.59	37.47	30.34		
Number	4	2	4		

Albumen Groups not Significantly Different
Anova: F 0.05 [4,9]=0.5819

Yolk Groups Significantly Different
Anova: F0.001 [4,36] =28.311

Embryo Groups Significantly Different
Anova. F0.001[3,24]=60.284

Shell groups not Significantly Different
Anova: F 0.005[2,7]=0.3705

correlation between the size of the female and the total number of eggs in the clutch. This was not supported in the present study but may simply reflect differences in sample sizes. Although there was a reasonable correlation (R^2=0.77) between egg diameter and the SCL of the female, the correlation between egg length and SCL was poor (R^2=0.18) (Parmenter, 1976). The generalization that egg length does not increase substantially with SCL but that egg diameter does was supported by the present results. The correlation between mean egg width and mean egg mass is associated with an increase in follicle diameter in larger females. Although not demonstrated in the present study, the increase in follicle diameter affects the diameter and increase in mass of the eggs in marine turtles (Miller, 1982). Because the eggs are oval and the yolks are round, there is little albumen between the vitelline membrane of the yolk and the inner portion of the shell membrane. The albumen is situated primarily towards the ends of the egg.

Embryonic growth in *Chelodina longicollis* as indicated by the change in dry mass of embryos and yolks is similar to that reported for *Chelydra serpentina* (Morris et al., 1983) and follows the general pattern of embryonic growth in marine turtles (Ackerman, 1981; Miller, 1982).

The distribution of calcium in fresh eggs of *Chelodina longicollis* is similar to that reported for other species of oviparous reptiles (see Packard and Packard, 1984). At oviposition the albumen contained only a small quantity of calcium. The yolk contained more, but less than the egg shell (Table 5). At the end of incubation, calcium reserves in the albumen had not been significantly reduced. Those of the yolk were reduced and calcium in the embryo had increased significantly.

The yolk contributed about 30% of the total calcium required by the developing embryo; the remaining 70% of the embryonic requirement was derived from the shell. This compares favorably with the contribution (% Ca) made by the egg shells of sea turtles (Cheloniidae and Dermochelyidae) of between 60 and 80 % (Simkiss, 1962, 1967; Bustard et al., 1969; Miller and Jones, unpub data). The contribution made by the egg shell of *Chelodina longicollis* is about 15 % higher than occurs in *Chelydra serpentina* (56%, M. Packard et al., 1984b) and is much higher than the contribution made by the poorly calcified egg shell of the snake *Coluber constrictor* (21 %, M. Packard et al., 1984a) and the lizard *Amphibolurus barbatus* (40 % M. Packard et al., 1985).

TABLE 6. Analysis of variance tables for calcium content and concentration in embryos, yolks, and the total egg (excluding shell) with initial egg weight as the potential covariate based on log (value + 1) NS=Not Significant.

Source	Sum of Squares	Degrees Freedom	Mean Square	F	P
Yolk Dry Weight (g)					
Day of incubation	4.841	9	0.538	33.92	0.01
Initial egg weight	0.809	1	0.809	51.04	0.01
Error	1.094	69	0.015		
Embryo Dry Weight (g)					
Day of incubation	2.719	9	0.302	14.44	0.01
Initial egg weight	0.226	1	0.226	10.02	0.01
Error	1.444	69	0.021		
Yolk Ca Concentration (mg/g)					
Day of incubation	18.563	9	2.063	11.84	0.01
Initial egg weight	0.0188	1	0.0188	0.11	NS
Error	12.022	69	0.174		
Embryo Ca Concentration (mg/g)					
Day of incubation	75.607	9	8.401	9.64	0.01
Initial egg weight	0.955	1	0.955	1.1	NS
Error	60.124	69	0.871		
Yolk Ca (mg)					
Day of incubation	44.307	9	4.923	135.4	0.01
Initial egg weight	1.830	1	1.830	50.34	0.01
Error	2.508	69	0.036		
Embryo Ca (mg)					
Day of incubation	112.512	9	12.501	184.17	0.01
Initial egg weight	0.625	1	0.625	9.21	0.01
Error	4.683	69	0.067		
Total Ca (mg) In Egg Excluding Shell					
Day of incubation	19.451	9	2.161	40.81	0.01
Initial egg weight	2.778	1	2.778	52.46	0.01
Error	3.654	69	0.053		

The total calcium in the entire egg (shell, albumen, yolk, embryo) apparently does not change but is redistributed. The total egg contains enough calcium for embryogenesis without obtaining any from the environment. However, the combined yolk and albumen cannot supply the requirements for embryogenesis without augmentation from the shell. The pattern of embryonic growth and incorporation of calcium indicates that the two sources (yolk, shell) are utilized more or less sequentially. More calcium is derived from the shell later in incubation as reserves in the yolk decline. This is consistent with the pattern of development of the allantois and indicates that extraction from the egg shell is by the chorioallontic membrane. Further, the two sources of calcium are not utilized equally by the embryo. By the end of incubation the calcium in the yolk was nearly exhausted; whereas the shell contained sufficient calcium to supply all the embryonic demand (based on samples taken early in incubation, Table 5).

The pattern of calcium utilization by embryonic *Chelodina longicollis* is similar to that reported for *Chelydra serpentina* (Packard et al., 1984a) and *Chrysemys picta* (Packard and Packard, 1986). The amount of calcium in the yolk is at first relatively stable suggesting little use of calcium by the embryo. However, the amount of yolk declines sharply after about 50

% of incubation. Similarly, there is no detectable change in calcium during the early stages of embryonic differentiation. However, the amount of calcium increases rapidly after the embryo begins the growth phase of development. The only noticeable variation in the two patterns occurs late in incubation of *Chelodina longicollis* when the rates of calcium uptake in the embryo and loss by the yolk is slow. This may be the case in reptilian eggs when the incubation period is variable. The incubation ranged between 63 and 82 days under constant conditions. This range is equal to one-third of the fastest developmental time and one-quarter of the slowest. At present the variation cannot be evaluated because few clutches were incubated, and the possibility of a subtle influence by minor temperature gradients cannot be discounted. There may also have been a minor influence resulting from forced oviposition, as has been shown in lizards (Jones, 1983).

Rapid mobilization of calcium into the embryo in the latter half of incubation coincides with the osteogenic phase of development. The primary use of calcium by the embryo is in the building of bones (Simkiss, 1967).

Embryonic calcium levels increase in concert with the decrease in yolk calcium. The yolk supplies the calcium necessary for the initiation of the embryonic growth phase. The somatic development of the embryo requires calcium (albeit small quantities) at a time when the allantois has not developed sufficiently to give ready access to the reserves in the shell. As the vitelline membrane extends around the yolk, the area between the sinus terminalis and the embryo becomes vascularised. This occurs close enough to the inner part of the shell membrane to allow the necessary respiratory exchange; only a thin layer of albumen and the chorion lie between them. This degree of apposition may allow some translocation of calcium. Certainly, as the area vasculosa of the vitelline increases, before the allantois extrudes between it and the chorion, some calcium may be acquired from the shell. However, at this time the demand is small and the vascularised surface of the vitelline is actively interacting with the yolk for the general nutrition of the embryo and apparently selectively removes calcium (see Packard et al., 1984a; Packard and Packard, 1984). The development of the allantois prior to (or simultaneously with) the increase in demand for oxygen and calcium ensures support for the growth phase.

The role of respiratory exchange in the translocation of calcium in reptilian eggs has not been demonstrated but gas exchange plays an important role in translocation during avian development (Crooks and Simkiss, 1974). Packard and Packard (1984) provide critical speculation about the function of the chorioallantois in translocation of calcium but no experimental data are available.

During incubation, embryos of domestic fowl (*Gallus domesticus*) store calcium derived from the shell in the yolk to such an extent that by hatching the yolk contains more calcium (50-75 %) and at a higher concentration than at oviposition (Johnston and Comar, 1955; Romanoff, 1967; Simkiss, 1967; Crooks and Simkiss, 1974). In contrast, the amount of calcium in yolks of *Chelydra serpentina* (Packard et al, 1984a), *Chrysemys picta* (Packard and Packard, 1986) and *Chelodina longicollis* decrease during incubation. The decrease in concentration of calcium in the yolk indicates that these embryos selectively remove calcium from the yolk. Simkiss (1967) reported a decline in the amount of calcium in yolks of *Caretta caretta* during incubation, although the temporal changes in the quantity and concentration were not determined; he also reported that the "calcium in the egg contents increases rapidly in the latter part of incubation and is five times greater at hatching than in the fresh egg" (Simkiss, 1967, p.229). Earlier, Simkiss (1962) demonstrated a fourfold increase of calcium within the egg of *Dermochelys coriacea*. Clearly these oviparous reptiles follow a similar pattern of utilization of calcium that is quite different from the pattern followed by birds.

Although all the calcium for embryogenesis is available from the yolk and shell, the ultimate source of calcium is the female who secretes both of these structures. Very little work has focused on this aspect of the overall role of calcium in reproduction. Data derived from a number of different reptiles (see review by Simkiss, 1967) provides only a partial picture. Reproduction by an oviparous reptile can be subdivided into two phases. The first phase includes the preparation of the follicles prior to ovulation. This may require only a month or so in some lizards or as long as two years or more (e.g. *Vipera berus*). The second phase is the deposition of the shell around the ovulated ova. The process of vitellogenesis provides the yolk proteins containing calcium over a period of time that is typically longer than the period required for deposition of the shell. The former does not

put as much stress on the calcium budget of the female as does the latter. The proximal source of calcium for vitellogenesis and shell deposition is bone (Dessauer and Fox, 1959) but the ultimate source is the diet of the female.

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